Identification and Sensitivity of Aerobic Bacteria Isolated from Urine of Chronic Renal Failure Patients.

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Dedication

To the soul of my Father , Mubarak and Yusuf with all my love

To my dear mother, sisters and family

To my friends and colleagues

To the renal failure patients to whom I wish speed recovery

Acknowledgement

First of all my thanks and praise to almighty Allah, the beneficent, the merciful, for giving me health and strength to accomplish this work. Then I would like to express my appreciation and sincere gratitude to my supervisor, Dr. Suliman Mohamed Elhassan for his keen guidance, valuable assistance, advice and moral encouragement during preparation of this study. Indeed, his generous help and support is greatly appreciated.

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My best regards to all without any exceptions.

Abstract

Sudan is a large country with about 39 million people and an annual incidence of chronic renal failure (CRF) of about 70-140 pmp/year. This study was conducted to evaluate the presence of urinary tract infection (UTI) and identify the bacteria commonly associated with this infection in Sudanese patients with CRF. Fifty patients were randomly selected from patients with CRF being treated by heamodialysis (HD) in the period from March 2006 to May 2007.

The study revealed that the male: female ratio was 1.9:1 and all of them were out patients. The prevalence of significant RBCs on urine was 42% and that of significant pyuria was 38%. The presence of bacterial infection on urine culture was 74%.

The commonest organism isolated was *Escherichia coli*, accounting for 42%.The other isolates were *Proteus mirabilis* 12% , *Pseudomonas aeruginosa* 6% , *Staphylococcus saprophyticus* 14% , *Staphylococcus caseolyticus* 14% , *Staphylococcus intermedius* 12% , and *Staphylococcus aureus* 4%.

Of the isolated organisms, 98% were sensitive to one or more of the tested antibiotics, and the best antibiotics of high effectiveness were Gentamycin (96.7%) and Nalidixic acid (70%).

المُســتخلــص

السودان بلد كبير المساحه ويقدر سكانه بحوالي 39 مليون نسمة وتقدر نسبة حدوث مرض الفشل الكلوى المزمن بحوالى 70-140 حاله لكل مليون نسمه سنويا. اجريت هذه الدراسة لمعرفة حدوث مرض انتان المجارى البولية والتعرف على البكتيريا المصاحبه لهذه الإنتانات فى مرضى الفشل الكلوى فى السودان.

أختير 50 مريضاً عشوائياً من بين مرضى الفشل الكلوى الذين يتلقون العلاج بالتنقية الدموية فى الفتره من مارس 2006 الى مايو 2007.

تبين من هذه الدراسة ان معدل الفشل الكلوى المزمن عند الرجال اعلى من النساء بنسبة 1,0:1,9 وكلهم مرضى خارجيين. وكان معدل وجود بيلة دموية معتده هو 42% و معدل وجود بيلة قيحية معتده هو 38%. كان معدل وجود بكتيريا بالبول عند تزريعه 74%.

كان اكثر الاحياء المجهريه التي تم عزلها الاشريكية القولونية حيث عزلت بنسبة 42%. كما تم عزل 12% من نوع البروتيس, و6% من نوع السيدوموناس, و14% من نوع المكورات العنقودية الرمية, و14% من نوع المكورات العنقودية المتجبنة, و12 % من نوع المكورات العنقودية الوسطية, و 4% من نوع المكورات العنقودية الذهبية.

كان 98% من الاحياء المجهرية التى تم عزلها حساسة لواحد او اكثر من المضادات الحيوية التى جربت و افضل المضادات فعاليه هى الجنتاميسين (96.7%) وحمض النالديكسيل(70%).

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections and it accounts for about 1-2 percent of all consultations (Davision and lambie, 1992). UTI can cause serious and permanent damage in patients with underlying urinary tract abnormalities (Martin *et al.*, 1992).

Patients with chronic renal failure (CRF) are more susceptible to develop UTI because of altered immunity, uraemia, low urinary flow rate and urinary concentration defect, all of which favor growth and multiplication of bacteria (Saitoh *et al*., 1985).

Furthermore, UTI can lead to more deterioration of renal function in patients with CRF due to many factors including septicemia, fluid depletion leading to hypotension and reduced cardiac output with reduced renal perfusion and obstruction to the urinary tract by sloughed papillae, stones or plugs of pus (Edwina, 1993).

Almost all cases of UTI are bacterial in origin, with Gram-negative enterobacteriaciae being the most common and *Escherichia coli* alone accounting for about 50-90% of cases (Edwina, 1993; Baker and Tomson, 1996). The remainders are due to proteus, pseudomonas, klebsiella, streptococci and staphylococci

In patients with CRF, regardless of the cause, there should be an active search for UTI both clinically and by laboratory investigations. Routine urinalysis is the first and the most important step in the investigation of such patients, followed by urine culture and sensitivity testing.

Hence the objectives of this study were to:

- Evaluate the presence of UTI among haemodialysis patients with CRF
- Identify the bacteria most commonly associated with these infections and their sensitivity to various antimicrobial agent

CHAPTER ONE

1. LITERATURE RIVEW

1.1 Chronic renal failure

The kidney's principal functions are excretion of metabolic waste products and the regulation of the volume and composition of body fluids.

The kidney is also responsible for the retention of substances vital to the body such as glucose, amino acids, phosphate and proteins. The kidney also plays an important role in endocrine and metabolic functions by the secretion of erythropoietin, rennin and prostaglandin and the metabolism of vitamin-D to active form.

Renal failure may be defined as failure of the renal excretory function due to depression of glomerular filtration rate.

Chronic renal failure (CRF) consists of persistent impairment of both glomerular and tubular function of gradual onset and of such severity that the kidneys are not longer able to keep the internal environment normal (Dewardener, 1998).

Acute renal failure (ARF) means a decrease in renal function lasting for days or weeks while CRF means renal impairment lasting month or years. ARF is more likely than CRF to be reversible depending on the cause; it may cause sudden life threatening, biochemical disturbances and is a medical emergency. CRF is often progressive, whatever the initial cause, because loss of nephrons lead to further destruction of the intact nephrons.

This progressive loss of renal function is due to several mechanisms including adaptive changes in glomerular haemodynamics, direct effect of hypertension on glomerular structure and function and phosphate retention (El-Nahas and Tamimi, 1998).

1.2 Epidemiology of chronic renal failure (CRF)

The annual incidence of epidemiology severe renal disease

(ESRD) varies in different countries. It is estimated to be about 25 per-million-populations (pmp) in developing countries, 28.6 pmp in Europe, 169 pmp in USA and 194.2 pmp in Japan (Davision, 1992).

The incidence of ESRD differs according to age, sex and race, being more common in males, higher in the elderly and in black people. The incidence of CRF is about 70- 140 pmp/year in Sudan, the majority being young patients below 40 years of age (Suliman *et al.*, 1995).

1.3 Haemodialysis

Blood from the patient is pumped through a semipermeable membrane which brings the blood into close contact with the dialysate flowing to the opposite direction. The rate of blood flow through the machine is maintained at 200-300 ml/minute and the rate of flow of dialysate is 500 ml/min. Movement of particles occurs along their concentration gradient. Haemodialysis (HD) is carried out through an arteriovenous fistula conducted surgically between the radial or brachial artery and the cephalic vein or an arteriovenous shunt or, in emergency cases, by a large bore, double lumen cannula inserted into a central vein. An average of 2-3 sessions of HD per week is adequate with duration of

4-5 hour each. Adequate HD maintains the body weight gain below 1.5 kg between dialysis sessions and maintains normal blood pressure, but the restriction of salt, water and proteins should be continued.

There is no known absolute contraindication for HD but relative contraindications include advanced malignancy, severe psychiatric illness, cerebrovascular event, myocardial infraction and elderly (Barry and Lazarus, 1991).

Complications of HD include clotting, bleeding or infection at the site of the fistula, peripheral neuropathy, hypotension, anaphylactic reactions, hard water syndrome, hemolytic reactions, air embolism, under dialysis syndrome, disequilibrium syndrome and transmission of infections, mainly hepatitis B and C and Human immune deficiency virus (HIV), for which routine screening is performed before dialysis and there are usually separate machines for hepatitis B virus /HBV positive patients.

The prevalence of hepatitis C virus/ (HCV) was estimated to be about 34.9% among Sudanese patients on HD and there was no correlation between the prevalence rate and blood transfusion and nosocomial transmission may be the main factor responsible for high prevalence rate (Suliman *et al.*, 1995). HCV infection is associated with considerable morbidity and isolation of seropositive patients is the best method to prevent transmission of HCV infection by HD (Padmanabham, 1994).

1.4 Urinary tract infection

1.4.1 Definition of urinary tract infection

Urinary tract infection (UTI) may be defined as microbial invasion of any of the tissues of the urinary tract from urethral orifice to the renal cortex (Richard and Reller, 1992). Normally, the urinary tract is sterile except for the distal third of the urethra which may contain a few commensals such as *Cintobacter s*pp and diphtheroids, yeasts may also be found in the female urethra (Monica, 1999).

Contamination of the urine with other commensals including streptococci, diphtheroids, and *Mycobacterium smegmatis* may occur as the urine specimen is being collected. In females' patients, the urine may become contaminated with organisms from the vagina and this often indicated by presence of epithelial cells and mixed bacterial flora (Suliman *et al.*, 1995).

1.4.2 Upper and lower urinary tract infection

Upper UTI indicates infection of kidney (pyelonephritis) while lower UTI indicates infection of the bladder, prostate and urethra.

Although the infection and resultant features may be related to one site, the presence of bacteruria places the entire UT at risk of invasion by bacteria (Richard and Reller, 1992).

1.4.3 Complicated and uncomplicated UTI

Uncomplicated UTI occurs in patients with functionally normal UT. It is usually of the lower UTI and is rarely associated with persistent renal damage.

Complicated UTI is the infection occurring in functionally abnormal UT such as obstructive uropathy or after instrumentation of the UT, or infection in patients with systemic diseases such as diabetes mellitus, immunosuppression, sickle cell disease, or analgesic nephropathy. It usually involves the kidney parenchyma (pyelonephritis) and is resistant to therapy, often result in relapse and lead to significant sequel such as sepsis, renal damage and metastatic abscesses (Richard and Reller, 1992).

1.4.4 Bacteruria

The presence of bacteria in urine is called bacteruria. Significant bacteruria is defined by Kass (1957) as the presence of 10 or more colony forming unit of bacteria per milliliter of urine on culture and it is a strong evidence of UTI, although lesser colony counts can be of diagnostic importance (Richard and Reller, 1992). A rigid adherence to the above definition should be avoided and culture counts should be interpreted in relation to the clinical information about the patient (Collee, 1996).

A colony count of 10 in asymptomatic patients can be taken as infection provided contamination is excluded. Contamination becomes more likely if there is a mixed growth and less likely if the same organism is cultured on more than one occasion or if the sample is taken from a male patient. Asymptomatic bacteruria indicates the presence of significant bacteruria without pyuria and in the absence of symptoms of UTI. It is especially common in women, being about 2-4 % in young and 10 % in elderly women (Suliman *et al.*, 1995). Asymptomatic bacteruria is rare in men but increases with advancing age and approaches the rate

in elderly women. In institutionalized elderly patients, the prevalence of asymptomatic bacteruria approaches 20-50 % in women and 5-20 % in men (Martin *et al*., 1992).

Screening for asymptomatic UTI is indicated in pregnancy because it occurs in up to 7 % of pregnant women and more than a quarter of patients later develop symptomatic UTI if left untreated (Tamimi and Mikhail, 1998). Continuing bacteruria is associated with premature delivery and increased prenatal mortality.

1.4.5 Pyuria

Pyuria indicates the presence of pus cells (white blood cells) in urine. Pus cells are usually reported as a number per high power field (HPF). Significant pyuria is defined as the presence of 10 or more pus cell per HPF of centrifuged urine (Tamimi and Mikhail, 1998).

Significant pyuria is a good evidence of UTI and its absence provide strong evidence against the presence of UTI. Sterile pyuria is the presence of significant pyuria with negative routine urine culture and it is due to the presence of fastidious organisms like *Mycobacterium tuberculosis*, other causes include analgesic nephropathy, perinephric abscess, disseminated fungal infection, and recent antibiotic therapy and UT stones ((Tamimi and Mikhail, 1998).

1.4.6 Recurrent urinary tract infection

Recurrence of UTI is the result of either relapse or reinfection and it is important to distinguish between them.

Reinfection is defined as eradication of bacteruria by appropriate treatment followed by infection with a different, or less commonly the same organism (Tamimi and Mikhail, 1998).

It usually occurs after 7-14 days and is due to reinvasion of UT and not due to treatment failure. It accounts for about 80 % of recurrent infection (Baker and Tomson, 1996).

Relapse is recurrence of infection with the same organism within 7 days of treatment and indicates failure to eradicate the infection (Martin *et al*., 1992). Most often occurs in association with renal scars, stones or cystic disease, prostatitis, chronic interstitial nephritis and in immunocomporomised patients (Tamimi and Mikhail, 1998).

1.5 Epidemiology of urinary tract infection

UTIs are one of the most common infections experienced by humans and are exceeded in frequency among ambulatory patients only by respiratory and gastrointestinal infection (Richard and Reller, 1992).

The annual general practice consultation rate for UTI in women is estimated to be 62.5 /1000 (Martin *et al.*, 1992), and the incidence of UTI in women is ten times that in men (Tamimi and Mikhail, 1998).

Chronic pyelonephritis, the end result of kidney infection, is responsible for about 30% of childhood and 20% of adult cases of end stage renal failure (Ascher, 1988). UTI is also an important cause of Gram-negative septicemia as about 20 % such cases result from infection in the UT (Ascher, 1988).

1.6 Pathogenesis of urinary tract infection

Spread of infection to UT may be via the blood steam, lymphatic or direct spread from an adjacent focus, but it is most often by the ascending transurethral route. The transurethral route involves three steps as following (Baker and Tomson, 1996).

- Periurethral colonization: this is facilitated by the adhesion of bacteria to uro-epithelial surface by pili and fimbriae. Previous episodes of UTI predispose to bacterial colonization. Other factors include lack of personal hygiene, wearing of nappies or sanitary towels and local infection.

- Transurethral passage of bacteria to the bladder; this is facilitated by catheterization, instrumentation or sexual intercourse. Spontaneous transfer along the short female urethra accounts for the increased incidence of UTI in women compared to men.

- Establishment and multiplication of bacteria in the bladder, the bladder urine is usually sterile due to the protective mechanisms of the bladder which include mucopolysaccharides, Tam-Horsfall proteins and urine flow and bladder contraction (Richard and Reller, 1992). A low flow rate and infrequent, and poor bladder emptying predispose infection.

Bladder bacteruria predisposes to the spread of infection to the kidneys. Protective renal factors against infection include high osmosllity, high ammonium concentration, phagocytosis, and urine flow (Baker and Tomson, 1996).

1.7 Bacteria associated with urinary tract infection

Organisms found in normal urine are staphylococci (coagulase negative), diphtheroids, bacilli and coliform bacteria. The importance pathogens are *E.coli* Proteus, Citrobacter, Pseudomonas, Klebsiella, Moraxella, Acintobacter, Staphylococcus, *Streptococcus faecalis, Salmonella* spp and *Mycobacterium tuberculosis* (Sleigh and Duguid, 1989).

1.7.1 *Escherichia coli*

Infection in the UT is directly related to the ability of bacteria to adhere and then colonize, first in the gut and then in the perineum and urinary tract in asending pattern. Adhesion of enterobacteriaceae is particularly important when pyelonephritis occurs in an anatomically normal. However, it also plays an important role in recurrent UTI and UTI complicating indwelling catheters (Langermann *et al*., 1997).

The virulence factors of *Escherichia coli*, the most common cause of UTI, are responsible for promoting the progression of the organism from the faecal reservoir to the bladder and occasionally to the kidneys. These factors include several properties pertaining to a small group of O-serotypes including O1, O2, O4, O6, O7, O16, O18 and O75 which are responsible for about 80% of cases of pyelonephritis, 60% of cystitis and 30% of cases of asymptomatic bacteruria (Meyner *et al*., 1998). *Escherichia coli* causes approximately 85% of cases of urethrocystitis (infection of the urethra and bladder), about 80% of cases of chronic bacterial prostatitis, and up to 90% of cases of acute pyelonephritis (inflammation of the renal pelvis and parenchyma). Approximately one

half of females have had a urinary tract infection by their late twenties due to *E coli* from their fecal flora (Foxman *et al*., 1995)

Adhesion on bacterial fimbriae (pili) and on bacterial surface recognized binding sites on the epithelial surface.Women who are not-secretors of histo- blood group antigens are more susceptible to recurrent UTI (Kuehn *et al*., 1992).

The presence of adhesions on *Escherichi coli* can be detected by phenotypic, genetic and molecular methods. In addition to adhesions, fimbriae have other virulence properties such as promotion of the persistence of infection, increasing the inflammatory response to infection and prevention of clearance of infection. The fimbriae have an important therapeutic role in preventing UTI such as the development of vaccines against these fimbriae which in turn inhibit *Escherichia coli* binding to epithelium of the UT (Langermann *et al*., 1997). Other factors implicated in the virulence of *E. coli* include the presence of flagellae which are necessary for motility, production of haemolysin for formation of pores in the cell membrane, secretion of aerobacteria for provision of iron to the bacteria and resistance to plasma bactericidal properties (Langermann *et al*., 1997).

1.7.2 *Klebsiella* spp

The main features of this group of lactose fermenting bacteria is that they are capsulated, non-motile and VP positive (Baker *et al*., 1980*). Klebsiella pneumoniae* is present in the respiratory tract and faeces of about 5% of normal individual and it occasionally produce UTI and bacteraemia with focal lesion in debilitated patients (Brooks *et al*.,1995).

Klebsiella and *Serratia* species commonly cause infections following intravenous and urinary catheterization and infections complicating burns. Klebsiella was first recognized clinically as an agent of pneumonia. *Klebsiella pneumoniae* accounts for a small percentage of pneumonia cases; however, extensive damage produced by the organism results in high case fatality rates (up to 90 percent in untreated patients) (Bingen, 1994).

1.7.3 *Proteus* spp

Proteus is rod shaped organisms, motile; spores and capsules are not produced (Buxton and Fraiser, 1994). Most proteus strains swarms with periodic cycles of migration producing concentric zones, the organism is methyl red positive, aceton negative, and proticin producer (Holt *et al*., 1994). *Proteus* species are part of the Enterobacteriaceae family of Gram-negative, *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora, *Proteus mirabilis* causes 90% of Proteus infections. The most common cause of brain abscesses in neonatal meningitis is *P mirabilis* and can be considered a community-acquired infection .The ability of Proteus organisms to produce urease and to alkalinize the urine by hydrolyzes urea to ammonia and carbon dioxide. Alkalinization of the urine by ammonia can cause magnesium phosphate and calcium phosphate to become supersaturated and crystallize out of solution to form, respectively, struvite and apatite stones. Bacteria within the stones may be refractory to antimicrobial therapy. Large stones may interfere with renal function (Dube *et al*., 1998). Proteus, particularly *P mirabilis*, is

believed to be the most common cause of infection-related kidney stones, one of the most serious complications of unresolved or recurrent bacteriuria *Proteus* species frequently causes nosocomial infections of the urinary tract, surgical wounds, and lower respiratory tract. Less frequently, *Proteus* species cause bacteremia, most often in elderly patients (Dube *et al*., 1998). The first step in the infectious process is adherence of the microbe to host tissue. Fimbriae facilitate adherence and thus enhance the capacity of the organism to produce disease. *E coli, P mirabilis*, and other Gram-negative bacteria contain fimbriae (ie, pili), which are tiny projections on the surface of the bacterium (Dembry and Andriole, 1997).

1.7.4 *Pseudomonas* spp

Pseudomonas species are slender rods, motile, aerobic, catalase and oxidase positive, some are capsulated, and has distinctive smell (Baker *et al*., 1980). Pseudomonades are resistant to many antimicrobial agents and therefore become dominant when more susceptible bacteria of the normal flora are suppressed (Brooks *et al*., 1998). *P. aeruginosa* have virulence factors that enable it to cause significant disease in the urinary tract. *P. aeruginosa* produces exotoxin A, endotoxins, hemolysins, and proteolytic enzymes. It is resistant to many antibiotics (Abbott, 2003). Of the two million nosocomial infections each year, 10% are caused by *P. aeruginosa*. The bacterium is the second most common cause of nosocomial pneumonia and the most common cause of intensive care unit (ICU) pneumonia. Pseudomonas infections can be spread within hospitals by health care workers, medical equipment, sinks, disinfectant

solutions, and food. These infections are a very serious problem in hospitals for two reasons. First, patients who are critically ill can die from a pseudomonas infection. Second, many Pseudomonas bacteria are resistant to certain antibiotics, which makes them difficult to treat (Dube *et al*., 1998). *P. aeruginosa* a pathogen associated with usage of intravenous or urinary catheters and with mucous membranes and skin damage (Brooks *et al*., 1998)

1.7.5 Enterobacter spp

Enterobacter spp. are Gram negative rods, motile, aerobic and facultatively anaerobic. *Enterobacter* spp. are found in water and soil, and are parasite of human intestinal tract. They have been isolated from septic conditions (Baker, 1980). A series of nationwide outbreaks of bacteremia (1970 to 1971 and 1973), caused by contaminated commercial fluids for intravenous injections, involved *Enterobacter cloacae* and *Enterobacter agglomerans* (Emori and Gaynes, 1993)

1.7.**6** ***Citrobacter*** **spp.**

Citrobacter spp. are straight rods, Gram negative, and motile. They are opportunistic pathogens and are occasionally isolated from urine, blood, pus, and other specimens (Holt *et al*., 1994). Citrobacter septicemia may occur in patients with multiple predisposing factors; *Citrobacter* species also cause meningitis, septicemia, and pulmonary infections in neonates and young children (Abbott, 2003). Total Enterotoxigenic strains of Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus also have been isolated from infants and children with acute gastroenteritis. The enterotoxins of at least some of these organisms are

of the heat-labile and heat-stable types and have other properties in common with the *E coli* toxins isolates from pregnant women (Civetta *et al*., 1997).

1.7.7 Staphylococci

Staphylococcus aureus is the primary pathogen in the genus Staphylococcus. *Staph. epidermidis* and *Staph. saprophyticus* are less commonly found as pathogens. They act, sometimes, as opportunistic pathogens and cause infection in the urinary tract in debilitated or immunodeficient subject, causing more serious bacteriaemic infection (Duguid, 1989).

Haemolysis is seen around most pathogenic colonies when grown on blood agar. *Staph. aureus* produce the enzyme coagulase nearly all strains of *Staph. epidermidis* lack this enzyme (Todar, 2002). About 70-90% of the population carry *Staph .aureus* in the nostrils at some time. Although present on the skin of only 5-20% of healthy people, as many as 40% carry it elsewhere, such as in the throat, vagina, or rectum. *S. aureus* flourishes in hospitals, where it infects healthcare personnel and patients who have had dialysis-dependent kidney disease. Staph. bacteria can also contaminate bedclothes, catheters, and other objects (Civetta *et al*., 1997)

1.7.8 Streptococci

Gram-positive cocci, arranged in pairs, short or long chains, can not form spores, and non motile. Many of the streptococci have a cell wall polysaccharide antigen called C substance which, when extracted by

hydrochloric acid or formamide by enzymes give an antigen used for their serological grouping (Lancefield groups) (Baker, 1980). *Streptococcus .faecalis* endocarditis usually responds to high dose of the combination penicillin and an amimoglycoside; vancomycin may be of value for strain that is particularly resistant to other drugs (Ross, 1989).

1.7.9 Enterococci

Enterococci are transmitted on medical devices. In patients the most common sites of infections are the urinary tract, wound, billiary tract and blood. In adults, enterococci can cause endocarditis. They grow at 45°C, ferment lactose, trehalose and sorbitol; but do not ferment inulin. They belong to Lancefeild group D (Holt *et al*., 1994).

Enterococci are resistant to sulphonamides and benzylepenicillin, but relatively sensitive to ampicillin and amoxicillin, which may be used in treatment of urinary tract infections. They are resistant to aminoglycosides (Ross, 1989).

1.8 Etiology of urinary tract infection

Although UTI may occasionally by caused by viruses and fungi, the overwhelming majority are caused by bacteria (Tamimi and Mikhail, 1998).

Gram- negative organisms cause the vast majority of cases and *Escherichia coli* alone causes about 50- 90% of all cases.

Other causative of Gram- negative organisms include *Klebsiella spp, Proteus spp, Pseudomonas, Enterococci* and *Citrobacter spp* (Richad and Reller, 1992).

Other common causes include *Staphylococcus saprophyticus* which is particularly common in sexually active women (Martin *et al*., 1992).

1.9 The role of urinary tract infection in chronic renal failure

A high incidence of UTI among patients with CRF has been reported. The underlying cause of chronic renal insufficiency might contribute to the increased incidence. Possible aetiologyical factors include the following:

Increased need for bladder catheterization for acute problems, severe oliguria with relation stasis throughout the UT which allows undisturbed proliferation of urinary pathogens and defects in the concentration ability of the kidneys interfering with the development of optimal concentrations of antimicrobial substances in urine (Saitoh *et al*., 1985).

Defects in both humeral and cell mediated immunities have also been encountered as cause of increased infections including UTI in patients with epidemiology of severe renal disease (ESRD). In these patients there are granulocytes with subnormal locomotion, phagocytosis and intracellular killing. Cell mediate immunity (CMI) is also depressed as evidenced by short lymphocyte survival, lymphopenia, inhibition of lymphocyte transformation and suppressor T-cell activity (Simeon and Reed, 1990).

Many studies were done worldwide to know the incidence of UTI in patients with CRF, mainly in patients on maintenance HD,and renal transplant recipients.

Kolendo *et al*. (1996) studied 118 patients with CRF on HD for infections and found that 32.4% patients have sepsis, 27.7% UTI, and 5.6% tuberculosis.

Saitoh *et al*. (1985) studied 182 patients with CRF for evidence of UTI by urinalysis and urine culture and found that 27% had significant bacteruria, 38% significant pyuria, 17% had UTI and 7% had asymptomatic UTI.

Chaurey *et al*. (1993) studied the occurrence of bacteruria and pyuria in asymptomatic HD patients and found that 31% had significant pyuria and 25% had significant bacteruria. They concluded that pyuria is a good marker of significant bacteruria in their patients and that the UT may represent a significant reservoir for infection.

UTIs are the most common bacterial infections in renal transplant recipient affecting about 30- 40% of patients (Rubin, 1993).

The major risk factors include indwelling catheters, handling and trauma to the kidney and ureter during surgery, anatomic abnormalities of the native or transplanted kidney, neurogenic bladder especially in diabetics' immunosuppression and rejection (Sayegh - Carpenter, 1998). The typical causative organisms are Gram- negative bacilli and enterococi, in addition to *Corynebacterium urealyticum* which is a newly recognized organism responsible for about 10% of cases of UTI in renal

transplant recipients while it causes only 2% of UTI in general population (Aguado *et al.*, 1993).

1.10 Treatment of urinary tract infection

The commonly used drugs for treatment of UTI are ampicillin, amoxicillin, trimethoprim or cotrimoxazol, aminoglycosides, cephalosporin, nitrofurantion, nalidixic acid, and recently, quinolones. Amoxicillin is no longer the first line antibiotic of chronic infection because of increased recurrence and fewer cure rate (Tamimi and Mikhail, 1998), but the addition of B-lactamase inhibitor (e.g. augmentin) is highly effective though expensive (Martin *et al.*, 1992).

CHAPTER TWO

2 MATERIAL AND METHODS

2.1 Sterilization

2.1.1 Flaming

It was used to sterilize glass slides, cover slips, needles, and scalpels, points of scissors and mouth of culture tubes by passing them through Bunsen burner flame without allowing them to become red hot**.**

2.1.2 Red heat

It was used to sterilize loop wires, points of forceps and searing spatulas by holding them over a Bunsen burner flame until they became red-hot.

2.1.3 Hot air oven

It was used to sterilize glassware such as bottles, flasks, test tubes, Petri dishes, Pasteur pipettes, graduated pipettes and also forceps. They were sterilized in hot air oven at 180 °C for one hour.

2.1.4 Steaming at 100 °C

Repeated steaming (Tyndallization) was used for the sterilization of sugars and media that could not be autoclaved without detrimental effect to their constituents. It was carried out as described by Cruickshank *et al.* (1975).

2.1.5 Moist Heat (autoclave)

Autoclaving at 121°C (151b/inch²) for 15 minutes was used for the sterilization of media and plastic wares. Autoclaving at 115°C (101b/ inch²) for 10 minutes was used for sterilization of some media.

2.2 Reagent and indicators

2.2.1 Reagents:

2.2.1.1 Hydrogen peroxide (H_2O_2)

This reagent was obtained from Agropharm limited, Buckingham. It was prepared as 3% aqueous solution and stored in dark and cool place. It was used for catalase test.

2.2.1.2 Kovac´s reagent

This reagent is composed of 5 g para-dimethylaminobenzaldehyde, 75 ml amyl alcohol and 25ml concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath. It was then cooled and acid was added. The reagent was stored at 4°C for later use in indole test.

2.2.1.3 Potassium hydroxide

This reagent was obtained from (BDH) and prepared as 4% aqueous solution for Voges-Proskauer test.

2.2.1.4 Methyl red solution

This was prepared according to Barrow and Feltham (1993) by dissolving 0.04 g of methyl red in 40 ml ethanol and the volume was made to 100 ml with distilled water. It was used for methyl red test.

2.2.1.5 Nitrate reagent

Nitrate test reagent was consisting of two solutions A and B. They were prepared according to Barrow and Feltham (1993). Solution A was composed of 0.33% sulphanilic acid dissolved by gentle heating in 5N acetic acid. Solution B was 0.6% composed of dimethylamine-alfa-naphthylamine dissolved by gentle heating in 5N acetic acid. Solution A and B were used for nitrate reduction test.

2.2.1.6 Alpha-naphthol solution

It was manufactured by British Drug House (BDH); London This reagent was prepared as 5% aqueous solution and was used for Voges-Proskauer (VP) test.

2.2.1.7 Tetra methyl-p-phenylene diamine dihydrochloride:

This was prepared in a concentration of 3% aqueous solution and was used for oxidase test

2.2.2 Indicators:

2.2.2.1 Andrade`s indicator

It was composed of acid fuchsin 5 g, distilled water 1 liter and N-Na OH 150 ml. The acid fuchsin was dissolved in distilled water, then the alkali solution was added, mixed and was allowed to stand at room temperature for 24 hours with frequent shaking until the color changed from red to brown. This was used for sugar fermentation test.

2.2.2.2 Bromothymol blue

It was obtained from BDH. It was prepared according to Barrow and Feltham (1993) by dissolving 0.2 g of powder in 100 ml distilled water. It was used for oxidation fermentation test.

2.2.2.3 Phenol red

It was supplied by Hopkins and William Lt, London. It was prepared as 0.2% aqueous solution. It was used for urea agar base medium.

2.2.2.4 Lead acetate paper

Filter paper strip, 4-5 mm wide and 50-60 mm long were impregnated in lead acetate saturated solution and then dried. It was used for hydrogen sulphide production test.

2.3 Collection of blood and plasma from laboratory animals

Blood for enriched media was collected aseptically into a sterile flask containing glass bead by venipuncture of jugular vein of healthy sheep kept for this purpose. The blood was defibrinated by shaking the sterile flask.

Blood for plasma used for coagulase test was aspirated directly from rabbit heart or an ear vein using a sterile vacutainer containing an anticoagulant ready prepared. Human plasma obtained from blood bank, Khartoum Hospital was also used for the test.

2.4 Preparation of media

2.4.1 Solid media

2.4.1.1 Blood agar (CM271)

Blood agar medium was composed of dehydrated blood agar base obtained from Oxoid LTd. and defibrinated sheep blood. The blood agar base contained heart infusion, tryptose, sodium chloride and agar. It was prepared according to manufacturer′ s instruction by dissolving 40 g in one liter of distilled water by boiling, and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 50°C, defibrinated sheep blood was added aseptically to give final concentration 10%, and mixed gently. Fifteen ml of complete medium was poured into each sterile Petri dish. The poured plates were allowed to solidify at room temperature on flat surface.

2.4.1.2 Nutrient agar (CM3)

Nutrient agar medium was obtained from Oxoid. It was composed of beef extract, peptone, sodium chloride and agar. The medium was prepared according to manufacturers by dissolving 28g of the powder in one liter of distilled water by boiling. Then the pH was adjusted to 7.3 and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 50°C and distributed in 15 ml amount per plate. The poured plates were left to solidify at room temperature on leveled surface.

2.4.1.3 MacConkey agar (CM7)

The dehydrated medium of MacConkey agar was composed of peptone; lactose, bile salts, sodium chloride, agar and 1% neutral red

.The medium was prepared according to manufacturer's (Oxoid) instructions by dissolving 52 g of MacConkey agar in 1 liter of distilled water, and boiled to dissolve the ingredients completely. Then the pH was adjusted to 8.0 and the medium was sterilized by autoclaving at 121°C for 15 minutes and poured into sterile Petri dishes in 15 ml amount. The poured plates were left to solidify at room temperature on the flat surface.

2.4.1.4 Urea Agar (CM 53)

The base medium of urea agar contained peptone, sodium chloride, dextrose, potassium dihydrogen phosphate, phenol red and agar. The medium was prepared according to manufacturer's (Oxoid) instructions by dissolving 2.4 g of powder in 95 ml distilled water by boiling and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 50 -55 °C and 5 ml of sterile (20%) urea solution was added aseptically. The medium was distributed in 10 ml amounts in sterile test tubes and allowed to set in inclined position.

2.4.1.5 Ammonium salt sugar

Ammonium salt sugar (ASS) was prepared as described by Barrow and Feltham (1993). One gram of $(NH_4)\ H_2PO_4$, 0.2g KCl, 0.2g $MgSO_4$. $7H_2O$, and 0.2g yeast extracts were added to 1 liter distilled water. The solids were dissolved by steaming, then the indicator bromothymol blue (0.04ml) was added and mixture was sterilized by autoclaving at 121°C for 15 min. The mixture was allowed to cool to about 60°C, then appropriate sterile carbohydrate solution was added to give final concentration of 0.5 – 1 %,and mixed. The complete medium

was distributed aseptically into sterile test tubes and allowed to solidify in inclined position.

2.4.1.6 Simmon´s citrate agar (CM 155)

Twenty three grams of Simmon´s citrate agar (Oxoid) were suspended in 1 liter of distilled water, dissolved by boiling and sterilized by autoclaving at 121°C for 15 minutes. The medium was poured aseptically into sterile McCartney bottles and allowed to solidify in inclined position.

2.4.1.7 Kligler`s iron agar (KIA)

This medium was obtained from Oxoid. It contained 20g casein enzyme hydrolysate 3g beef extract, 3g yeast extract, 5g sodium chloride, 10g lactose, 1g glucose, 0.2g ferrous sulphate, 0.3 sodium thiosulphate, 0.25g phenol red and 15.5 agar. It prepared by dissolving 57.7g of dehydrated media in 1L distilled water, by boiling, cooled to 50 °C. Then the pH was adjusted to 7.4. The medium was then sterilized by autoclaving at 121°C for 15 minutes and poured in sterile bottles aseptically and allowed to set in inclined position.

2.4.1.8 Triple sugar iron agar (CM 277):

This medium was obtained from Oxoid. It contained 3g meat extract, 3g yeast extract, 20g peptone, 1g glucose , 10g lactose , 10g sucrose, 0.2g FeSO .2 H_2O, 0.3 g NaSO . 5HO and 20 g agar. The solid were dissolved in 1 liter distilled water by heating. Twelve ml 0.2% phenol red solution were added, mixed and dispensed into tubes. The medium was then sterilized by autoclaving at 121°C for 15 minutes and cooled to form

slope with deep butt. It was used for detection of hydrogen sulphide production.

2.4.1.9 Mannitol salt agar:

One hundred and eleven grams of Oxoid CM85 dehydrated medium were suspended in a liter of distilled water, steamed to dissolve and the pH was adjusted to 7.5. It was then autoclaved at 121°C for 15 minutes, cooled and poured into Petri-dishes. The poured plates were allowed to solidify at room temperature on flat surface

2.4.2 Semi solid media

2.4.2.1 Hugh and Leifson´s (O/F) medium

The medium was composed of dipotassium hydrogen phosphate, peptone, sodium chloride, agar and 0.2 % aqueous solution of bromocresol purple. The medium was prepared as described by Barrow and Feltham (1993). Two grams of peptone powder, 5 g of sodium chloride, 0.3 g. of potassium hypophosphate and 3 g of agar were added to 1 liter of distilled water then heated in water bath at 55°C to dissolve the solids. The pH was adjusted to 7.1 and filtered. The indicator bromothymol blue (0.2 % aqueous solutions) was added and the mixture was sterilized by autoclaving at 115 °C for 15 minutes. Filtered sterile glucose solution was added aseptically to give final concentration of 1% .Then the medium was mixed and distributed aseptically in 10 ml amount into sterile test tubes of no more than 16 mm diameter.

2.4.2.2 Motility medium

Motility medium was prepared as described by Barrow and Feltham (1993). It consisted of 10 g peptone, 3 g meat extracts, 5g sodium chloride, 4g agar, 80 g gelatin and 1 liter distilled water. First gelatin was soaked in water for 30 minutes then the other ingredients were added. The pH was adjusted to 7.4. This medium was dispended in 5 ml volume into 20 ml test tubes containing the appropriate Cragie tubes, and then the medium was sterilized by autoclaving at 121°C for 15 minutes.

2.4.3 Liquid media

2.4.3.1 Nutrient broth (B 274)

This medium contained, beef extract, peptone and sodium chloride. Thirteen grams of nutrient broth were added to 1 L of distilled water according to Barrow and Feltham (1993), and mixed well. Then the pH was adjusted to 7.2-7.4 and the medium was distributed in 3 ml amount into clean test tubes, and then sterilized by autoclaving at 121°C for 15 minutes.

2.4.3.2 Peptone water (CM 9)

This medium contained peptone and sodium chloride. It was prepared according to Barrow and Feltham (1993) by dissolving 10 g of peptone and 5 g of sodium chloride in 1 L of distilled water and mixed well. Then the pH was adjusted to 7.2 - 7.4 and the medium was distributed in 3 ml amount into clean test tubes and sterilized by autoclaving at 121°C.

2.4.3.3 Peptone water sugar

Peptone water sugar medium was prepared as described by Barrow and Feltham (1993). It contained peptone water 900 ml, Andrade's indicator 10 ml, sugar 10 g and distilled water 90 ml. The pH of peptone water was adjusted to 7.1-7.3 before the addition of Andrade´s indicator. The indicator was added to the mixture of peptone water and, mixed well. The complete medium was then distributed into portion of 2 ml into sterile test tubes containing inverted Durham's tubes, covered with metal caps and sterilized by autoclaving at 115°C (101b/inch^2) for 10 minutes. The sterilized medium was kept at 4°C until used.

2.4.3.4 Glucose phosphate (MR – VP) medium (CM 43)

This medium was prepared according to Barrrow and Feltham (1993). Peptone 5g and 5g of phosphate buffer (K_2HO_4) were added to one liter of distilled water, steamed till dissolved, filtered and pH was adjusted to 7.5. Then 5 g of glucose were added, mixed well, distributed into clean test tubes and sterilized by autoclaving at 115 °C for 15 min.

2.4.3.5 Nitrate broth medium

This medium was prepared according to Barrow and Feltham (1993). Potassium nitrate 1g was dissolved in 1 liter nutrient broth and the pH was adjusted to 8.0 – 8.4 and distributed into clean test tubes and sterilized by autoclaving at 115 °C for 20 minutes.

2.5 Collection of samples

Fifty samples were collected randomly from chronic renal failure patients, who visited Haemodialysis Center of Khartoum and Bahry hospital for routine haemodialysis, they were asked to submit sample of urine and they were instructed to collect mid stream specimens of urine in sterile universal bottles. The samples were put on ice in thermos flasks and transported immediately to the laboratory for immediate processing and culturing.

2.6 Microscopic examination of the urine

Ten ml of urine samples were centrifuged at 500 rpm for 15 minutes the supernatant was then decanted and the deposit was used for preparation of wet mount**s.** A drop of urine deposit was placed onto a clean glass slide, covered with a cover slip and examined under the microscope for the presence of pus cell, RBC, yeast cell and cast cell per field using the dry high power objective lens. The presence of 5-7 or more cell of pus and RBC was considered a significant pyuria and significant RBC in urine.

2.7 Isolation

The collected urine samples were inoculated onto 10% defibrinated sheep blood agar and MacConkey agar. The inoculated plates were then incubated aerobically at 37°C for 24 hours as described by Barrow and Feltham (1993). Further incubation was continued for another 24 h and if no growth was evident, then the plates were discarded as negative.

After the incubation period, the colonies characteristics were observed, smears were made from each type of colony. The smear was stained by Grams technique.

2.8 Purification

All bacterial isolates were purified by several subculturing from single well-separated colony on nutrient agar plates. The purity was checked by examining Gram stained smear. The pure culture was then used for studying cultural and biochemical characteristics and sensitivity of the isolates.

2.9 Identification of isolates

2.9.1 Microscopic examination

Smears were made from purified colonies, fixed by heating and stained by Gram stain method of Barrow and Feltham (1993). Then examined microscopically for cell morphology and arrangement, and staining reaction. Gram stain was also used to check the purity.

2.9.2 Cultural characteristics

All cultures on solid media were examined with naked eye for growth and colonial morphology and any changes in medium. Blood agar was used as an enriched, non-inhibiting medium for primary isolation of bacteria and for determination of colonial morphology and hemolytic activity.

The liquid media were also examined with naked eye for turbidity, color change, formation of sediments and accumulation of gas in the Durham's tube in case of carbohydrates media.

29.3. Biochemical tests

2.9.3.1 Sugar fermentation test

The test was carried out as described by Barrow and Feltham (1993). The peptone water sugar was inoculated with organism under the test, incubated at 37°C and then examined daily for several days. Acid production was indicated by appearance of reddish color, while gas production was indicated by presence of an empty space in the inverted Durham's tubes.

2.9.3.2 Oxidase test

The method of Barrow and Feltham (1993) was followed. Strips of filter paper was soaked in 1% solution of tetramethyl-p-phenylenediamine dihydrochloride and dried in hot air oven and then placed on clean glass slide by sterile forceps. A fresh young test culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper strip. If a purple color developed within 5-10 seconds, the reaction was considered positive.

2.9.3.3 Catalase test

The test was carried out as described by Barrow and Feltham (1993). A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean glass slide. A colony of test culture on nutrient agar was

picked off and put on the drop of hydrogen peroxide. Evolution of gas and appearance of bubbles indicated positive test.

2.9.3.4 Coagulase test

The test was performed as described by Barrow and Feltham (1993). To 0.5 ml of 1:10 dilution of human plasma in saline, 0.1 ml of 18-24 h old broth culture of test organism was added, then incubated at 37°C and examined after 6-24 h for coagulation. Definite clot formation indicated positive result.

The test was also performed as described by Barrow and Feltham (1993) on slide. Two colonies of tested culture were placed on a clean glass slide, emulsified in a drop of normal saline and then a loopful of human plasma was added to the drop of bacterial suspension. Appearance of coarse visible clump was recorded as positive result.

2.9.3.5 The Oxidation-fermentation (O/F) test

The test was carried out as described by Barrow and Feltham (1993). Duplicate test tubes of Hugh and Leifeson´s medium were inoculated with test organism by stabbing with straight wire. To one of the test tube a layer of sterile melted soft paraffin oil was added to depth of 3 cm above the medium to seal it from air. The inoculated tubes were incubated at 37°C and examined daily for fourteen days. Yellow color in open tube only indicated oxidation of glucose, yellow color in both tubes indicated fermentation reaction and blue or green color in open tube and green color in the sealed tube indicated production of alkali.

2.9.3.6 Indole production test

Indole production test was carried out as described by Barrow and Feltham (1993). The test organism was inoculated into peptone water and incubated at 37°C for 48 h. One milliliter of the Kovac´s reagent was run down along side of test tube. Appearance of pink color within a minute indicated positive reaction.

2.9.3.7 Methyl red (MR) test

Methyl red test was carried out as described by Barrow and Feltham (1993). The test organism was inoculated into glucose phosphate medium (MR-VP), then incubated at 37°C for 48 h. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction, whereas orange or yellow color indicated negative reaction.

2.9.3.8 Voges-Proskauer (VP) test

The test was performed as described by Barrow and Feltham (1993). The test culture was inoculated into glucose phosphate medium (MR-VP), then incubated at 37°C for 48 h. Three milliliter of 5% alpha-naphthol solution and 1 ml of 40% potassium hydroxide were added. When bright pink color developed within 30 minutes, the reaction was regarded as positive.

2.9.3.9 Nitrate reduction

The nitrate test was carried out as described by Barrow and Feltham (1993).The test culture was lightly inoculated into nitrate broth and incubated at 37°C for two days. Then 1 ml of solution A followed by

1 ml of solution B of nitrite test reagent were added. Red color indicated positive reaction that showed nitrate in the medium had been reduced to nitrite. If red color did not develop, powdered zinc was added to see whether there was residual nitrate or not. Red color development indicated that nitrate in medium had been reduced to nitrite by zinc but not by organism, whereas unchanged color indicated nitrate in original medium had been reduced completely and nitrite was further broken down by organism.

2.9.3.10 Urease activity tests

The test was carried out as described by Barrow and Feltham (1993).

The test organism was inoculated heavily onto slope urea agar medium and incubated at 37°C for two days. Appearance of red color indicated positive reaction.

2.9.3.11 Citrate utilization

The test was performed as described by Barrow and Feltham (1993).

The test culture was inoculated as a single streak over the surface of slope of Simon's citrate medium and examined daily for 7 days. Growth of the organism and change of color to pink indicated positive test.

2.9.3.12 Hydrogen sulphide (H_2S) production

The method of Barrow and Feltham (1993) was followed. The test culture was inoculated into nutrient broth; filter paper impregnated with 10% lead acetate solution was placed in the neck of the tube and incubated at 37ºC for two days. Brown or black color of the paper indicated positive reaction.

2.9.3.13 Ammonium salt sugar test

The test was performed as described by Barrow and Feltham (1993). The test organism was inoculated onto slope of ASS medium and incubated at 37°C for up to 7 days. The medium was examined on alternative days for growth and acid production.

2.9.3.14 Motility test

The Craigi tube in semi-solid nutrient agar prepared as described by Cruckshank *et al*. (1975) was inoculated. A small piece of the colony of the bacterium under test was picked by the end of the straight wire and stabbed in the center of semi solid agar in the Craigi tube and then incubated at 37°C overnight. The organism was considered motile if there was turbidity in the medium in/outside the Craigi tube.

2.9.3.15 Novobiocin sensitivity test

Standard disc diffusion method was used to carry out the sensitivity of the test organism to the antibiotic (5mg novobiocin sensitivity disc).

A plate of nutrient agar was dried in the incubator for 30 minutes then a diluted suspension of an over- night culture of the organism was poured onto the surface of the medium. Excess fluid was aspirated and the plate was allowed to dry again for 30 minutes. Using sterile forceps, the antibiotic disc was gently applied on the plate and incubated at 37°C for 24 hours. The zone of growth inhibition was measured in millimeters and reported as, sensitive 17 mm or more, moderately sensitive 15-16 mm and resistant 14 mm or less.

2.10 Sensitivity to antibiotics

The antibiotics sensitivity of bacterial strains isolated in this study were tested by diffusion technique (Cruickshank *et al*., 1975). The test bacterium was grown in peptone water and incubated at 37°C for two hours. The Petri dishes containing Mueller Hinton medium was dried in oven at 40°C for 20 minutes, then 1-2 ml of the culture was poured on it. The inoculum was distributed and a pipette was used to withdraw off the excess fluid. The plate was left to dry at room temperature for 15 minutes. Commercial disc obtained from Oxoid (Ltd.) were placed on the surface of the inoculated Mueller Hinton agar medium. Then incubated aerobically at 37°C for 16-18 hours. After incubation, the plates were examined for growth inhibition around the disc and diameter of zone of growth inhibition was measured in millimeters.

CHAPTER THREE

3 RESULTS

3.1 Survey

Fifty samples were collected randomly from HD patients with chronic renal failure in Khartoum and Bahry HD centers. The samples were collected from 33 male, and17 female (Fig 1).

The urine sample were subjected to general and bacteriological examination by direct microscopy and culture

3.2 Direct microscopic examination of specimens

Direct microscopic examination was used for detection of pus and red blood cells (RBCs). Significant pyuria were observed in 19 samples (38%) as shown in Fig 3 and RBCs in 21 samples (42%) (Fig 4), but yeast cell and cast cell was not been detected (0%)

3.3 Bacterial isolation

All samples were cultured onto Blood and MacConkey agar medium and incubated aerobically at 37°C. Thirty seven samples (74%) revealed growth of bacteria, while 13 (26%) samples did not revealed growth of bacteria as shown in Fig 2.

3.4 Characterization and identification of isolates

The bacterial isolates obtained in this study were identified according to their cultural characteristic, cell morphology, Gram stain reaction and their biochemical properties as described by Barrow and Feltham (1993).

The total number of bacterial isolates was 52, 30 were Gram negative isolates and 22 were Gram positive isolates (Table, 1).

3.5 Gram-negative bacteria

Thirty (60%) isolate of Gram-negative bacteria were isolated in this study. They consist of 21 (42%) *Escherichia coli*, 6 (12%) *Proteus spp*, and 3 (6%) *Pseudomonas* spp as shown in Table 2.

3.5.1 *Escherichia coli*

The twenty one (42%) *Escherichia coli* isolated in this study represent the highest percentage of isolation. These isolates were Gram-negative rods, motile, catalase positive, indole positive, and produced acid and gas from glucose, lactose, maltose, mannitol, and xylose (Table, 4).

3.5.2 *Proteus spp*

The six (12%) isolates of *Proteus* spp obtained in this study were *Proteus mirabilis*. These isolates were Gram-negative rod, motile, catalase positive, oxidase negative, and attacked sugar fermentatively with varied reactions in adenitol (Table, 4).

3.5.3 *Pseudomonas* spp

The three (6%) Pseudomonades isolated in this study were *Pseudomonas aeruginosa*. These were Gram-negative rods, motile, catalase positive, oxidase positive with characteristic pigmentation on nutrient agar, and they produced acid from glucose, fructose, glycerol, mannitol and xylose (Table, 4).

3.6 Gram positive bacteria

The total number of Gram-positive bacteria was 22 (44%) of samples examined (Table, 3). They were further characterized as *Staphylococcus spp*

3.6.1 *Staphylococcus* spp

Twenty two strains of *Staphylococcus* spp were isolated in this study. They were 7(14%) *Staphylococcus saprophyticus*, 7 (14%) *Staphylococcus caseolyticus*, 6 (12%) *Staphylococcus intermidis* and 2(4%) *Staphylococcus aureus* (Table, 3).These isolates were Gram-positive bacteria, non motile, non spore forming and fermented a number of sugars as shown in table 5. When *Staphylococcus aureus* was cultured on blood agar and incubated at 37°C for 24 hours, a wide zone of beta hemolysis was produced around the colonies.

3.7 Antibiotics sensitivity test

The bacteria isolated in this study were subjected to antimicrobial susceptibility testing by disc diffusion method on Mueller Hinton medium using commercial antibiotics discs.

3.7.1 Sensitivity of *Escherichia coli* to antibiotics

The sensitivity of *Escherichia coli* isolated in this study varied between 100%-76.2% to Gentamycin, Nalidixic acid and Nitrofurantion (Table,6) and showed resistance to Ampicillin, Cotrimoxazol, Colistin and Tetracycline that varied from 66.6% -42% as shown in Table 7 and Fig 5.

3.7.2 Sensitivity of *Proteus* spp to antibiotics

The *Proteus* spp isolated in this study were highly sensitive to Gentamycin 100% and Streptomycin 83.3% (Table, 6) and (Fig, 5) and showed resistance to Tetracycline and Nitrofurantion as shown in Table 7.

3.7.3 Sensitivity of *Pseudomonas* spp to antibiotics

The *Pseudomonas aerugenosa* isolated in this study revealed high resistance (100%) to six antimicrobial agents tested in this study (Table,

7) and showed intermediate sensitivity to Gentamycin (66.6%) and low sensitivity to Streptomycin 33.3% as shown in Fig 6.

3.7.4 Sensitivity of *Staphylococcus* spp to antibiotics

The *Staphylococcus* spp isolated in this study were highly sensitive to Gentamycin 100% and sensitivity to Erythromycin, Cloxcillin and Chloroamphencol varied from 100%-85.7% (Table, 8) and (Fig, 6). (Table, 9) *Staphyloccous* spp showed high resistance 100% to Tetracycline and from 100%-85.7% resistance to Erthromycin, Cloxcillin and Chloroamphencol as shown in Fig 6.

Table (1) Bacterial species isolated from urine samples collected from HD patient with renal failure in Bahry and Khartoum HD center

Bacterial species	Number isolated	Percentage % *	Isolation frequency**
Escherichia coli	21	40	42%
Proteus mirabilis	6	11.6	12%
Pseudomonas aeruginosa	3	6	6%
Staphylococcus saprophyticus	7	13.5	14%
Staphylococcus caseolyticus	7	13.5	14%
Staphylococcus intermedius	6	11.6	12%
Staphylococcus aureus	2	3.8	4%
Total	52	100	104%

* Calculated from the total number of isolates
** Calculated from total number of examined samples

Table (2) Gram-negative bacteria isolated from urine samples collected from HD patient with renal failure in Bahry and Khartoum HD center

Bacterial species	Number isolated	Percentage *	Isolation frequency**
Escherichia coli	21	70	42%
Proteus mirabilis	6	20	12%
Pseudomonas aeruginosa	3	10	6%
Total	30	100	60%

* Percentage % from the total number of Gram -ve isolates
** Calculated from total number of examined samples

Table (3) Gram positive bacteria isolated from urine samples collected from HD patient with renal failure in Bahry and Khartoum HD center

Bacterial species	Number isolated	Percentage % *	Isolation frequency**
Staphylococcus saprophyticus	7	32	14%
Staphylococcus caseolyticus	7	32	14%
Staphylococcus intermedius	6	27	12%
Staphylococcus aureus	2	9	4%
Total	22	100	44%

* Percentage % from the total number of Gram+ve isolates
** Calculated from total number of examined samples

Table (4): Characters and biochemical properties of Gram-negative bacteria isolated from urine samples collected from HD patients in Khartoum and Bahry HD center

Character	*Escherichia coli*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
Shape	Rod	Rod	Rod
Motility	Motile	Motile	Motile
Oxidase	+	-	+
Catalase	+	+	+
O/F	F	F	-
Growth on MacConkey	+	+	+
Citrate utilization	+	+	+
Urease	-	+	+
Indole	+	-	-
H_2S production	-	+	-
MR(37)	+	+	-
VP(37)	-	-	-
KIA: Slope Butt H_2S Gas	 Y Y - +	 R Y + -	
Acid from:			
Glucose	+	+	+
Lactose	+	-	-
Maltose	+	-	-
Mannitol	+	-	+
Salicin	V	-	-
Sorbitol	+	-	-
Sucrose	V	+	-
Trehalose	+	+	+
Xylose	+	+	+
Raffinose	+	+	+
Adenitol	V	V	V
Dulictol	V	+	V
Rhamnose	+	-	-

+ = positive Y = Yellow O = Oxidative F = Fermentative
- = Negative R = Red V = Variable

Table (5): Characters and biochemical properties of Gram-positive bacteria isolated from urine samples collected from HD patients in Khartoum and Bahry HD center

Character	*Staphylococcus Aureus*	*Staphylococcus saprophyticus*	*Staphylococcus caseolyticus*	*Staphylococcus intermedius*
Shape	S	S	S	S
Gram reaction	+	+	+	+
Aerobic growth	+	+	+	+
Motility	-	-	-	-
Catalase	+	+	+	+
Oxidase	-	-	-	-
O/F	F	F	F	F
Coagulase	+	-	-	-
Novobiocin	S	R	S	S
VP	+	+		-
Urease	+	+	-	+
Nitrate	+	-	+	+
Acid from:				
Mannose	+	-		+
Sucrose	+	+	-	+
Mannitol	+	+	+	+
Lactose	+	+	+	+
Fructose	+	+		+
Trehalose	+	+	+	+
Glucose	+	+	-	-
Maltose	+	+	-	-
Xylose	-	-	-	-
Raffinose	-	-	+	-

+ = positive - = Negative F = Fermentative

O = Oxidatve S = Sensitive R = Resistant

Table (6) Antibiotic sensitivity of Gram- negative bacteria isolated from HD patient with renal failure in Bahry and Khartoum HD center

Bacteria species	No of isolates examined	Number (percentage) of isolates sensitive to :							
		AMP	COT	GEN	NAL	NIT	COL	STR	TET
Escherichia coli	21	7(33.0%)	8(38.0%)	21(100.0%)	18(85.7%)	16(76.2%)	11(52.4%)	13(61.9%)	12(57.1%)
P .mirabilis	6	3(50.0%)	4(66.6%)	6(100.0%)	3(50.0%)	2(33.3%)	3(50.0%)	5(83.3%)	2(33.3%)
Pseudomonas aeruginosa	3	0(00.0%)	0(00.0%)	2(66.6%)	0(00.0%)	0(00.0%)	0(00.0%)	1(33.3%)	0(00.0%)
Total	30	10(33.3%)	12(40%)	29(96.7%)	21(70%)	18(60%)	14(46.7%)	19(63.3%)	14(46.7%)

AMP: Ampicillin 25 ug, COT: Cotrimoxazole 25 ug, GEN : Gentamycin 10 ug, NAL: Nalidixic acid 30 ug, NIT: Nitrofurantion 200 ug, COL: Colistin 30 ug, STR: Streptomycin 10 ug, TET: Tetracycline 10 ug.

Table (7) Antibiotics resistance of Gram- negative bacteria isolated from HD patient with renal failure in Bahry and Khartoum HD center

Bacteria species	No of isolates examined	Number (percentage) of isolates resistant to :							
		AMP	COT	GEN	NAL	NIT	COL	STR	TET
Escherichia coli	21	14(66.6%)	13(61.9%)	0(00.0%)	3(14.2%)	5(23.8%)	10(47.6%)	8(38.0%)	9(42.8%)
P .mirabilis	6	3(50.0%)	2(33.3%)	0(00.0%)	3(50.0%)	4(66.6%)	3(50.0%)	1(16.6%)	4(66.6%)
Pseudomonas aeruginosa	3	3(100.0%)	3(100.0%)	1(33.3%)	3(100.0%)	3(100.0%)	3(100.0%)	2(66.6%)	3(100.0%)
Total	30	20(66.7%)	18(60%)	1(3.3%)	9(30%)	12(40%)	16(53.3%)	11(36.7%)	16(30%)

AMP: Ampicillin 25 ug, COT: Cotrimoxazole 25 ug, GEN : Gentamycin 10 ug.,NAL: Nalidixic acid 30 ug, NIT: Nitrofurantion 200 ug, COL: Colistin 30 ug, STR: Streptomycin 10 ug, TET: Tetracycline 10 ug.

Table (8) Antibiotics sensitivityof Gram- positive bacteria isolated from HD patient with renal failure in Bahry and Khartoum HD center

Bacteria species	No of isolates examined	Number (percentage) of isolates sensitive to :							
		AMP	CHL	CXC	ERY	GEN	PEN	STR	TET
Staphylococcus saprophyticus	7	0(00.0%)	6(85.7%)	7(100.0%)	7(100.0%)	7(100.0%)	0(00.0%)	6(85.7%)	0(00.0%)
Staphylococcus caseolyticus	7	0(00.0%)	7(100.0%)	6(85.7%)	6(85.7%)	7(100.0%)	1(14.2%)	5(71.4%)	0(00.0%)
Staphylococcus intermedius	6	1(16.6%)	6(100.0%)	6(100.0%)	6(100.0%)	6(100.0%)	0(00.0%)	5(83.3%)	0(00.0%)
Staphylococcus aureus	2	0(00.0%)	1(50.0%)	1(50.0%)	1(50.0%)	2(100.0%)	2(100.0%)	1(50.0%)	0(00.0%)
Total	22	1(4.5%)	20(90.9%)	20(90.9%)	20(90.9%)	22(100%)	3(13.6%)	17(77.3%)	0(00.0%)

MP: Ampicillin 25 ug, CHL: Chloroamphencol 10 ug, CXC: Cloxcillin 5 ug, ERY: Erythromycin 5 UG, GEN : Gentamycin 10 ug, PEN: Penicillin 10 ug, STR: Streptomycin 10 ug, TET: Tetracycline 10 ug.

Table (9) Antibiotic resistance of Gram- positive bacteria isolated from HD patient with renal failure in Bahry and Khartoum HD center

Bacteria species	No of isolates examined	Number (percentage) of isolates resistant to :							
		AMP	CHL	CXC	ERY	GEN	PEN	STR	TET
Staphylococcus saprophyticus	7	7(100.0%)	1(14.2%)	0(00.0%)	0(00.0%)	0(00.0%)	7(100.0%)	1(14.2%)	7(100.0%)
Staphylococcus caseolyticus	7	7(100.0%)	0(00.0%)	1(14.2%)	1(14.2%)	0(00.0%)	6(85.7%)	2(28.5%)	7(100.0%)
Staphylococcus intermedius	6	5(83.3%))	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	6(100.0%)	1(16.6)	6(100.0%)
Staphylococcus aureus	2	2(100.0%)	1(50.0%)	1(50.0%)	1(50.0%)	0(00.0%)	0(00.0%)	1(50.0%)	2(100.0%)
Total	22	21(95.4%)	2(9.09%)	2(9.09%)	2(9.09%)	0(00.0%)	19(86.4%)	5(22.7%)	22(100.0%)

AMP: Ampicillin 25 ug, CHL: Chloroamphencol 10 ug, CXC: Cloxcillin 5 ug, ERY: Erythromycin 5 UG, GEN : Gentamycin 10 ug, PEN: Penicillin 10 ug, STR: Streptomycin 10 ug, TET: Tetracycline 10 ug.

Table (10) Antibiolic sensitivity of Gram- negative and G-positive bacteria isolated from HD patient with renal failure in Bahry and Khartoum HD center

Type of bacteria	Number of isolates examined	Number (percentage %) of isolates sensitive to:											
		AMP	GEN	STR	TET	COT	NAL	NIT	COL	PEN	ERY	CXC	CHL
G-	30	10(33.3)	29(90.9)	19(63.3)	14(46.7)	12(40)	21(70)	18(60)	14(46.7)				
G+	22	1(4.5)	22(100)	17(77.3)	0(00.0)					3(13.6)	20(90.9)	20(90.9)	20(90.9)

AMP: Ampicillin 25 ug, GEN : Gentamycin 10 ug, STR: Streptomycin 10 ug, TET: Tetracycline 10 ug, COT: Cotrimoxazole 25 ug, NAL: Nalidixic acid 30 ug, , NIT: Nitrofurantion 200 ug, , COL: Colistin 30 ug, PEN: Penicillin 10 ug, ERY: Erythromycin 5 UG, CXC: Cloxcillin 5 ug, CHL : Chloroamphencol 10 ug.

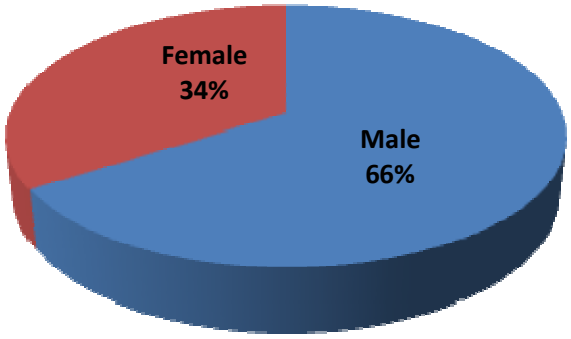


Fig 1: Sex distribution of HD patient with CRF in Bahry and Khartoum Hospitals

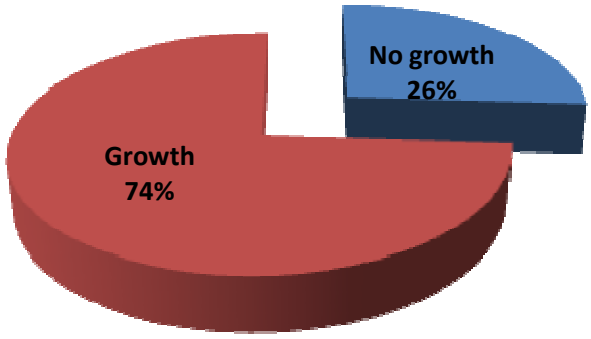


Fig 2: Bacterial growth from urine samples collected from HD patient with CRF in Bahry and Khartoum Hospitals

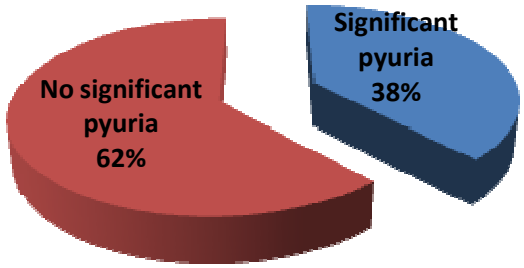


Fig 3: WBC presence urine wet mount of haemodialysis patient in Bahry and Khartoum Hospitals

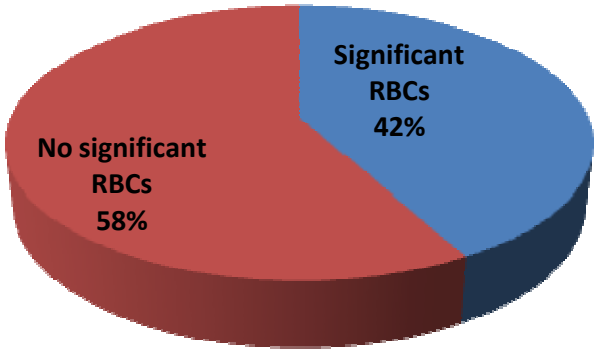


Fig 4: RBCs presence in urine wet mount of haemodialysis patient in Bahry and Khartoum Hospitals

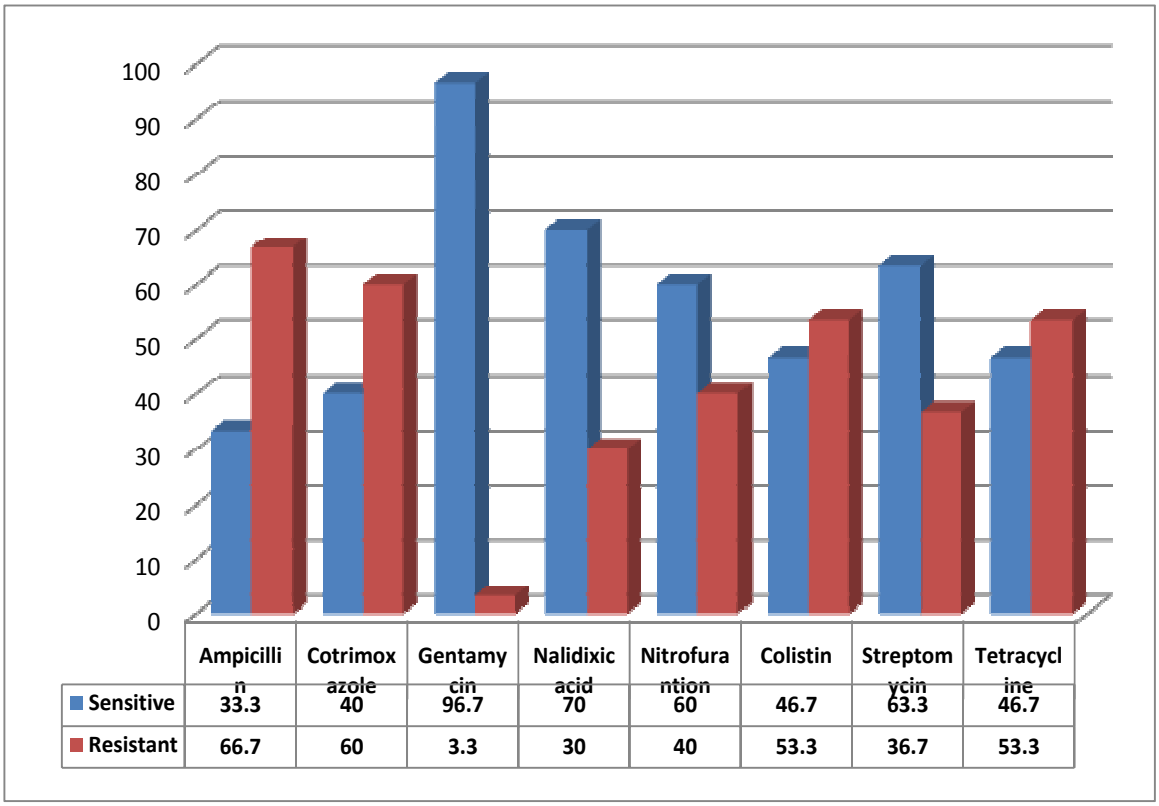
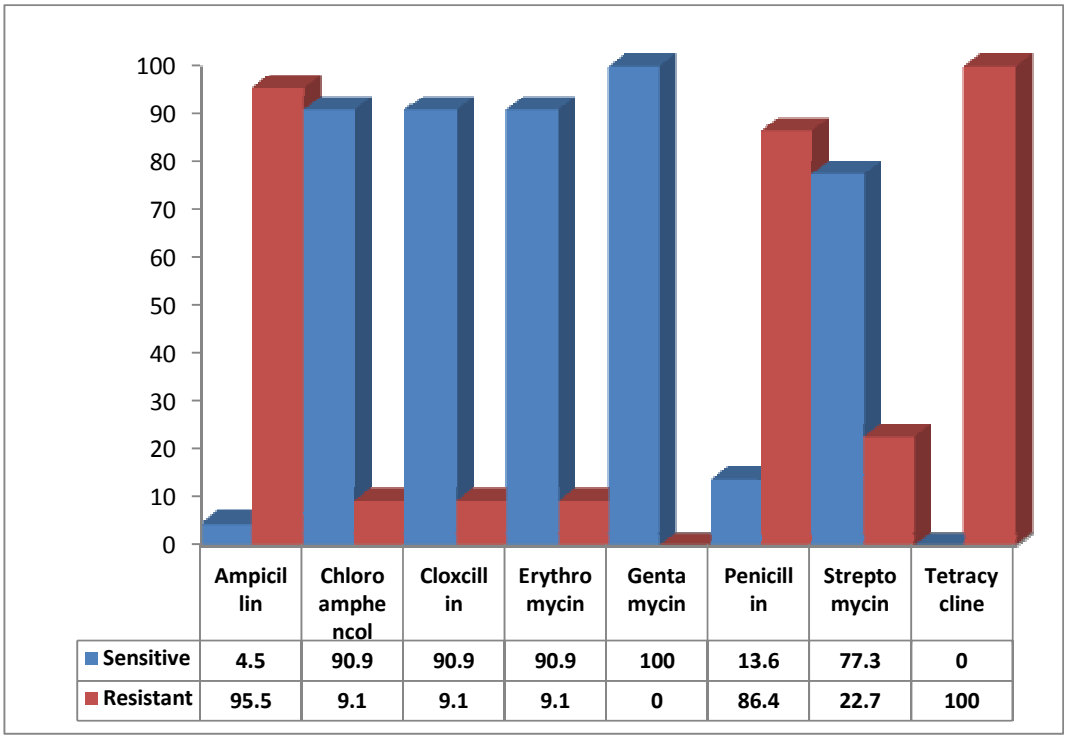


Fig 5: Antibiotic sensitivity of Gram-negative bacteria isolated from urine samples collected from haemodialysis patients in haemodialysis centers in Bahry and Khartoum Hospitals



	Ampicillin	Chloramphenicol	Cloxcillin	Erythromycin	Gentamycin	Penicillin	Streptomycin	Tetracycline
Sensitive	4.5	90.9	90.9	90.9	100	13.6	77.3	0
Resistant	95.5	9.1	9.1	9.1	0	86.4	22.7	100

Fig 6: Antibiotic sensitivity of Gram-positive bacteria isolated from urine samples collected from haemodialysis patients in haemodialysis centers in Bahry and Khartoum Hospitals

CHAPTER FOUR

4. Discussion

The present study was designed to evaluate the presence of UTI and identify the bacteria commonly associated with the infection in Sudanese patient with CRF.

In this study thirty seven samples (74%) revealed bacterial growth and the rest thirteen samples (26%) did not show any bacterial growth. These results indicate that 74% of urinary tract infections in haemodialysis patients were infected with bacteria. This is agreed with Kolendo *et al*. (1996) who studied 118 patients with CRF on HD for infections and found that 32.4% patients have sepsis, 27.7% UTI, and 5.6% tuberculosis.

In this study the prevalence of significant RBCs on urine 42% and that of significant pyuria was 38%. This is almost similar to that obtained by Saitoh *et al*. (1985) who studied 182 patients with CRF for evidence of UTI by urinalysis and urine culture and found that 27% had significant bacteruria, 38% significant pyuria, 17% had UTI and 7% had asymptomatic UTI. Also Chaurey *et al*. (1993) studied the occurrence of bacteruria and pyuria in asymptomatic HD patients and found that 31% had significant pyuria and 25% had significant bacteruria. They concluded that pyuria is a good marker of significant bacteruria in their patients and that the UT may represent a significant reservoir for infection.

Species of bacteria isolated from urine samples in this study were mainly enterobacteria, constituting, 54%. This percentage is lower than that obtained by Hussein (2002) and Ibrahim (1994) who reported enterobacteria isolation as 77.9% of total isolates and EL-sheikh (2004) who isolated enterobacteria as 67.9% of total isolates. Foxman *et al*. 1995 stated that coliforms and Proteus were responsible for 46% of urinary tract and 24% of surgical site infections, 17% of the bacteremias, and 30% of the pneumonias from 1990 through 1992. *Escherichia coli*, the predominant nosocomial pathogen, is the major cause of infection in the urinary tract and is common in other body sites

Escherichia coli isolation constituted 42% of the total isolates in this study. Ibrahim (1994) found *E.coli* represented 45.4% of total isolates from urine of patients living in urban and rural areas and similar to EL-sheikh (2004) findings which showed *E.coli* constituted 42.9% of total isolates from pregnant women. Foxman *et al*. (1995) found that *Escherichia coli* causes approximately 85 percent of cases of urethrocystitis (infection of the urethra and bladder), about 80 percent of cases of chronic bacterial prostatitis, and up to 90 percent of cases of acute pyelonephritis (inflammation of the renal pelvis and parenchyma). Approximately one half of females have had a urinary tract infection by their late twenties due to *E coli* from their fecal flora

Proteus mirabilis isolation rate in this study constituted 12% of urine samples examined. Hussein (2002) isolated *Proteus mirabilis* from 4% of urine samples. On other hand, Ibrahim (1994) isolated this species from 6.8% of urine samples examined. Mohammed (2003) isolation of

Proteus mirabilis represented 16.6% of the total isolates from urine of males. EL-sheikh (2004) isolated this species from 7.1% of pregnant women urine samples. The isolation rate of *Pseudomonas aeruginosa* was 6% in this study. However Kabinde *et al*. (2004) reported isolation of 34.4% *Pseudomonas aeruginosa* of total isolates from burn infections.

Staphylococci were isolated from 44% of urine examined samples in this study. This finding was higher than that of Ibrahim (1994) who reported the isolation of 14.2% from urine and higher than that of Hussein (2002) who reported the isolation of 24% out of the total bacteria isolated from urine samples of diabetic females. Mohammed (2003) reported higher isolation which constituted 73% of the total isolates from urine of males.

The isolation of *Staphylococcus* species from 44% out of total urine sample in this study was almost similar to 41.66% reported by Wadi (1986) but higher than 10.3% reported by Rastegar *et al*. (2005). Emori and Gaynes (1992) reported that *Staphylococcus aureus* accounts for only 0.5% to 6% of all positive urine cultures. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are currently the most common pathogens in nosocomial pneumonias, followed by Enterobacter and Klebsiella (Emori and Gaynes, 1992).

About 70-90% of the populations carry *Staph. aureus* in the nostrils at some time. Although present on the skin of only 5-20% of healthy people, as many as 40% carry it elsewhere, such as in the throat,

vagina, or rectum, for varying periods of time, from hours to years, without developing symptoms or becoming ill (Civetta et al., 1997).

From the findings of this study and pervious one it can be concluded that *E coli* and *Saphylococcus* species are the prime cause of UTI in Sudan, other organisms causing UTI varied in their percentage.

In this investigation, Gentamicin was highly effective against *Staphylococcus aureus* while Ampicillin and Tetracycline were not effective against *Staphylococcus aureus.* This study agrees with Abdalhameed (1999) who reported highly resistant of *Staphylococcus* spp to Penicillin (97.9%). Also agreed with Andrews (2001) who reported of Cholaramphenicol, Ampicillin and Tetracyciline resistance. It should be noted that although all uropathogens isolated in this study were sensitive to gentamicin, however it is not suitable for use in-patients with UTI (Andrews, 2001). It was observed, that, the *Staphylococcus* species isolated from urine samples in this study, were highly resistance to the most commonly used antibiotics (Ampicillin, Tetracycline and penicillin) in Sudan

Kennth (2004) reported that *Staphylococcus aureus* is one of the major resistant pathogens found on the mucous membranes and the skin of around a third of the population, it is extremely adaptable to antibiotic pressure. It was the first bacterium in which penicillin resistance was found to in 1947, just four years after the drug started being mass-produced. Methicillin was then the antibiotics of choice. Methicillin-resistance *Staphylococcu aureus* (MRSA) was first detected in Birtain in

1961. Half of all *S.aureus* infections in the US are resistant to Penicillin, Methicillin, Tetracycline and Erythromycin (Kennth, 2004).

All strains of *Pseudomonas aeruginosa* isolated in this study were resistance to Ampicillin (100%), Cotrimoxazole (100%), Nalidixic acid (100%), Nitrofurantion (100%), Colistin (100%), Tetracycline (100%), Streptomycin (66.6%) and Gentamicin (33.3%) as illustrated in Table 7. These results agreed with that obtained by Abdelhameed (2005) who reported completely resistance of *Pseudomonas aeruginosa* to Penicillin, Cloxacillin, Methicillin, Clindamycin, Sulphonamides, Vancomycin and Erythromycin.

The sensitivity of *E.coli* strains isolated in this study to Gentamicin, Streptomycin, Nalidixic acid, Nitrofurantion and Tetracyciline varied between 85.7% -57.1% (Table 6), while they showed low sensitivity to Ampicillin and Cotrimoxazole (33%). This result agreed with Beck *et al*. (1994) who reported that resistance of *E.coli* to Ampicillin, and first generation Cephalosporins is increasing rapidly to the extent that they can not longer be considered primary drugs of choic in empirical treatment of urinary tract infections.

The *Proteus mirabilis* isolated in this study were highly sensitive to Gentamicin (100%) and Streptomycin (83.3%) and show variable sensitivity to other antibiotics as illustrated in Table 6 and they show less sensitivity to Ampicillin (40%) and this result disagrees with Kaye (1998) who reported that most strains of *Proteus mirabilis* are sensitive to Ampicillin and Cephalosporins.

Conclusions and Recommendations

Conclusions:

From the findings of this investigation it can be concluded that:

1. Male: female ratio was 1.9: 1. All of them were out patients
2. Many (38%) of the urine samples revealed presence of pus cell
3. Twenty one samples revealed presence of red blood cells (RBCs).
4. Most of patients (74%) in the study had positive bacterial growth when urine was cultured.
5. *E.coli* being the most common organism isolated 40%.
6. Almost all 96.7% of the isolated organisms were sensitive to Gentamycin. Nalidixic acid was the second most sensitive antibiotics in this study (70%)
7. Sensitivity to the commonly used antibiotics, such as Ampicillin and Cotrimoxazole was very low.

Recommendations

Routine urine culture should be done to every patient with CRF and UTI before, during and after treatment to enable the assessment of response to treatment and detection of recurrence**.**

There should be an active search for the detection of UTI in patients with CRF with proper management of those having UTI to prevent further deterioration of renal function in those patients.

The population should be aware of the problems of the UTI and it's squallier through health education and should be advised to seek medical care early, when they have symptoms referred to the urinary tract, particularly those with known predisposing factors.

The best antibiotics with high sensitivity which can be used as first line drugs are Gentamycin and Nalidixic acid.

Further studies should be conducted to find out the sensitivity of drugs not tested in this study such as Cephalosporin and quinolones.

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